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(54) Title: HYDROXAMIC ACID DERIVATIVES			
(57) Abstract <p>Compounds of general formula (I) wherein X is -CONHOH or -CO₂H, principally characterised by the presence in substituent R₃ and/or R₄ of a group of formula (II) are inhibitors of matrix metalloproteinases and inhibitors of the production of tumour necrosis factor.</p>			
<div style="text-align: center;"> <p style="text-align: right;">(I)</p> </div> <div style="text-align: center;"> <p style="text-align: right;">(II)</p> </div>			

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Hydroxamic Acid Derivatives.

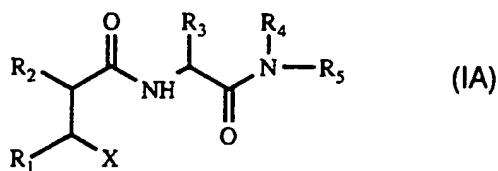
The present invention relates to therapeutically active hydroxamic acid and carboxylic acid derivatives, to processes for their preparation, to pharmaceutical compositions containing them, and to the use of such compounds in medicine. In particular, the compounds are inhibitors of metalloproteinases involved in tissue degradation, and in addition are inhibitors of the release of tumour necrosis factor from cells.

Background to the Invention

Compounds which have the property of inhibiting the action of metalloproteinases involved in connective tissue breakdown such as collagenase, stromelysin and gelatinase (known as "matrix metalloproteinases", and herein referred to as MMPs) are thought to be potentially useful for the treatment or prophylaxis of conditions involving such tissue breakdown, for example rheumatoid arthritis, osteoarthritis, osteopenias such as osteoporosis, periodontitis, gingivitis, corneal epidermal or gastric ulceration, and tumour metastasis, invasion and growth.

Metalloproteinases are characterised by the presence in the structure of a zinc(II) ionic site. It is now known that there exists a range of metalloproteinase enzymes that includes fibroblast collagenase (Type 1), PMN-collagenase, 72 kDa-gelatinase, 92 kDa-gelatinase, stromelysin, stromelysin-2 and PUMP-1 (J.F. Woessner, FASEB J, 1991, 5, 2145-2154). Many known MMP inhibitors are peptide derivatives, based on naturally occurring amino acids, and are analogues of the cleavage site in the collagen molecule. A recent paper by Chapman et al J. Med. Chem. 1993, 36, 4293-4301 reports some general structure/activity findings in a series of N-carboxyalkyl peptides. Other known MMP inhibitors are less peptidic in structure, and may more properly be viewed as pseudopeptides or peptide mimetics. Such compounds usually having a functional group capable of binding to the zinc (II) site in the MMP, and known classes include those in which the zinc binding group is a hydroxamic acid, carboxylic acid, sulphhydryl, and oxygenated phosphorus (eg phosphinic acid and phosphonic acid) groups.

Two known classes of pseudopeptide or peptide mimetic MMP inhibitors have a hydroxamic acid group or a carboxylic group respectively as their zinc binding groups. With a few exceptions, such known MMPs may be represented by the structural formula (IA)



in which X is the zinc binding hydroxamic acid (-CONHOH) or carboxylic acid (-COOH) group and the groups R₁ to R₅ are variable in accordance with the specific prior art disclosures of such compounds. Examples of patent publications disclosing such structures are given below.

In such compounds, it is generally understood in the art that variation of the zinc binding group and the substituents R₁, R₂ and R₃ can have an appreciable effect on the relative inhibition of the metalloproteinase enzymes. The group X is thought to interact with metalloproteinase enzymes by binding to a zinc(II) ion in the active site. Generally the hydroxamic acid group is preferred over the carboxylic acid group in terms of inhibitory activity against the various metalloproteinase enzymes. However, the carboxylic acid group in combination with other substituents can provide selective inhibition of gelatinase (EP-489,577-A). The R₁, R₂ and R₃ groups are believed to occupy respectively the P1, P1' and P2' amino acid side chain binding sites for the natural enzyme substrate. There is evidence that a larger R₁ substituent can enhance activity against stromelysin, and that a (C₁-C₆)alkyl group (such as iso-butyl) at R₂ may be preferred for activity against collagenase whilst an alkylphenyl group (such as phenylpropyl) at R₂ may provide selectivity for gelatinase over the other metalloproteinases.

Pseudopeptide or peptide mimetic MMP inhibitors of formula (IA) with potent *in vitro*

activities are known, but are generally poorly absorbed following oral administration. Although, it is known that a number of factors can influence oral absorption (such as aqueous solubility, pKa, log P and molecular weight) the design of pseudopeptide enzyme inhibitors with high oral absorption is far from straightforward. Finding a combination of R₁, R₂, R₃, R₄ or R₅ substituents that permits a good balance of intrinsic level of activity, water solubility, oral absorption, and pharmacokinetic properties is a continuing problem in the art, since those properties can vary in an unpredictable way as the substituents R₁ - R₅ are varied. Identifying hydroxamic and carboxylic acid-based MMP inhibitors having such properties remains a much sought after goal in the art.

Tumour necrosis factor (herein referred to as "TNF") is a cytokine which is produced initially as a cell-associated 28kD precursor. It is released as an active, 17kD form, which can mediate a large number of deleterious effects in vivo. When administered to animals or humans it causes inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase responses, similar to those seen during acute infections and shock states. Chronic administration can also cause cachexia and anorexia. Accumulation of excessive TNF can be lethal.

There is considerable evidence from animal model studies that blocking the effects of TNF with specific antibodies can be beneficial in acute infections, shock states, graft versus host reactions and autoimmune disease. TNF is also an autocrine growth factor for some myelomas and lymphomas and can act to inhibit normal haematopoiesis in patients with these tumours.

Compounds which inhibit the production or action of TNF are therefore thought to be potentially useful for the treatment or prophylaxis of many inflammatory, infectious, immunological or malignant diseases. These include, but are not restricted to, septic shock, haemodynamic shock and sepsis syndrome, post ischaemic reperfusion injury, malaria, Crohn's disease, mycobacterial infection, meningitis, psoriasis, congestive heart failure, fibrotic disease, cachexia, graft rejection, cancer, autoimmune disease, rheumatoid arthritis, multiple sclerosis,

radiation damage, toxicity following administration of immunosuppressive monoclonal antibodies such as OKT3 or CAMPATH-1 and hyperoxic alveolar injury.

Since excessive TNF production has been noted in several diseases or conditions also characterised by MMP-mediated tissue degradation, compounds which inhibit both MMPs and TNF production may have particular advantages in the treatment or prophylaxis of diseases or conditions in which both mechanisms are involved.

Recently, WO 93/20047 disclosed a class of hydroxamic acid based MMP inhibitors which also are active in inhibiting TNF production.

As mentioned above MMP inhibitors have been proposed with hydroxamic acid or carboxylic acid zinc binding groups. The following patent publications disclose hydroxamic acid-based MMP inhibitors:

US 4599361	(Searle)
EP-A-0231081	(ICI)
EP-A-0236872	(Roche)
EP-A-0274453	(Bellon)
WO 90/05716	(British Bio-technology Ltd ("BBL"))
WO 90/05719	(BBL)
WO 91/02716	(BBL)
WO 92/09563	(Glycomed)
EP-A-0497192	(Roche)
WO 92/13831	(BBL)
WO 92/17460	(Smithkline Beecham)
EP-A-0489577	(Celltech)
EP-A-0489579	(Celltech)
WO 92/22523	(Research Corporation Technologies)
US 6256657	(Sterling Winthrop)
WO 93/09090	(Yamanouchi)

WO 93/09097	(Sankyo)
WO 93/20047	(BBL)
WO 93/21942	(BBL)
WO 93/24449	(Celltech)
WO 93/24475	(Celltech)
EP-A-0574758	(Roche)
EP-A-0575844	(Roche)
WO 94/02446	(BBL)
WO 94/02447	(BBL)

The following patent publications disclose carboxylic acid-based MMP inhibitors:

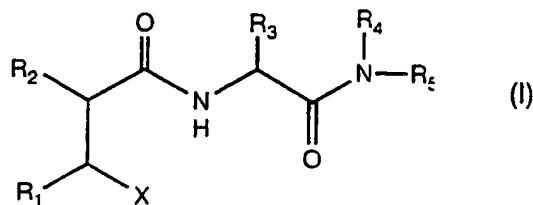
EP-A-0489577 (Celltech)
EP-A-0489579 (Celltech)
WO 93/24449 (Celltech)
WO 93/24475 (Celltech)

Brief Description of the Invention

This invention makes available a novel group of compounds of formula (IA), wherein X is a hydroxamic acid or carboxylic acid group, whose novel characterising feature is carbohydrate moiety in one or both of the groups R₃ and R₄. The compounds of the invention have good intrinsic activity as inhibitors of the enzymes collagenase, gelatinase and stromelysin, and inhibit the production of the pro-inflammatory cytokine TNF. The compounds also have useful pharmacokinetic properties, including bioavailability after oral administration.

Detailed Description of the Invention

According to a first aspect of the invention there is therefore provided a compound of general formula (I):



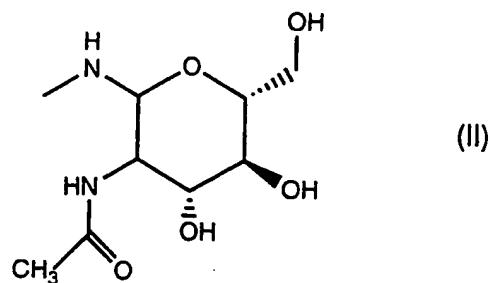
wherein;

X is -CONHOH or -CO₂H;

R₁ is hydrogen, a C₁-C₆ alkyl, C₂-C₆ alkenyl, phenyl, substituted phenyl, phenyl(C₁-C₆ alkyl), heterocycll, substituted heterocycll, heterocycll(C₁-C₆ alkyl), substituted heterocycll(C₁-C₆ alkyl), or a group BSO_nA- wherein n is 0, 1 or 2 and B is hydrogen or a (C₁-C₆) alkyl, phenyl, substituted phenyl, heterocycll, C₁-C₆ acyl, phenacyl or substituted phenacyl group, and A represents C₁-C₆ alkyl; amino; protected amino; acylamino; OH; SH; C₁-C₆ alkoxy; C₁-C₆ alkylamino; C₁-C₆ alkylthio; aryl(C₁-C₆ alkyl); amino(C₁-C₆ alkyl); hydroxy(C₁-C₆ alkyl), mercapto(C₁-C₆ alkyl) or carboxy(C₁-C₆ alkyl) wherein the amino-, hydroxy-, mercapto- or carboxyl-group are optionally protected or the carboxyl- group amidated; lower alkyl substituted by maleimido, succinimido, naphthalimido, 2,3-dihydro-1,3-dioxo-1H-benz[d,e]isoquinol-2-yl, carbamoyl, mono(lower alkyl)carbamoyl, di(lower alkyl)carbamoyl, di(lower alkyl)amino, carboxy-lower alkanoylamino, pyrrolidino or morpholino;

R₂ is a (C₁-C₆)alkyl, (C₂-C₆)alkenyl, (C₂-C₆)alkynyl, phenyl(C₁-C₆)alkyl, heteroaryl(C₁-C₆)alkyl, cycloalkyl (C₁-C₆)alkyl or cycloalkenyl (C₁-C₆) alkyl group anyone of which may be optionally substituted by one or more substituents selected from (C₁-C₁₀)alkyl, O(C₁-C₆) alkyl, S(C₁-C₆) alkyl, O(C₁-C₆ alkyl)OC₁-C₆ alkyl, S(C₁-C₆ alkyl)OC₁-C₆ alkyl, O(C₁-C₆ alkyl)SC₁-C₆ alkyl or S(C₁-C₆ alkyl)SC₁-C₆ alkyl;

R₃ is (a) a group -(C₁-C₆ alkyl)COR₆, or -(C₁-C₆ alkyl)(C₆H₄)XR₆ where X is a group -OCH₂CO, -CO-, -CH₂CH₂CO- or -NHCH₂CO- and R₆ is a group of formula (II):



or (b) (subject to the proviso below) the side chain of a naturally occurring amino acid, which may be protected if functional groups are present, eg by acylation of amino groups and amidation of carboxyl groups; or a group $CR_8R_9R_{10}$ in which each of R_8 , R_9 and R_{10} is independently hydrogen, (C_1 - C_6)alkyl, (C_2 - C_6)alkenyl, (C_2 - C_6)alkynyl, phenyl(C_1 - C_6)alkyl, hydroxyl, $O(C_1$ - $C_6)$ alkyl, $S(C_1$ - $C_6)$ alkyl, OPh , OCH_2Ph , SPh , SCH_2Ph , halogen, CN, CO_2H , (C_1 - C_4)perfluoroalkyl, CH_2OH , $CO_2(C_1$ - $C_6)$ alkyl, or a group phenyl or heteroaryl which is optionally substituted by one or more substituents independently selected from hydrogen, hydroxyl, halogen, CN, CO_2H , $CO_2(C_1$ - $C_6)$ alkyl, $CONH_2$, $CONH(C_1$ - $C_6)$ alkyl, $CONH(C_1$ - C_6 alkyl)₂, CHO, CH_2OH , (C_1 - C_4)perfluoroalkyl, $O(C_1$ - $C_6)$ alkyl, $S(C_1$ - $C_6)$ alkyl, $SO(C_1$ - $C_6)$ alkyl, $SO_2(C_1$ - $C_6)$ alkyl, NO_2 , NH_2 , $NH(C_1$ - $C_6)$ alkyl, $N((C_1$ - $C_6)$ alkyl)₂, $NHCO(C_1$ - $C_6)$ alkyl, (C_1 - C_6)alkyl, (C_2 - C_6)alkenyl, (C_2 - C_6)alkynyl, (C_3 - C_8)cycloalkyl, (C_4 - C_8)cycloalkenyl, phenyl or benzyl; or R_8 and R_9 together with the carbon atom to which they are attached form a 3 to 8 membered cycloalkyl or a 5- to 6-membered heterocyclic ring; or R_8 , R_9 and R_{10} together with the carbon atom to which they are attached form a bicyclic ring (for example adamantyl);

R_4 is (i) a group $-(CH(R_7)CONH)_mCOR_6$, wherein $m = 0$, 1, or 2, R_6 is as defined above, and R_7 is hydrogen or the side chain of a naturally occurring amino acid, which may be protected if functional groups are present, eg by acylation of amino groups and amidation of carboxyl groups;

or (ii) (subject to the proviso below) hydrogen, C₁-C₆ alkyl, (C₁-C₄)perfluoroalkyl or a group D-(C₁-C₆ alkyl) wherein D represents hydroxy, (C₁-C₆)alkoxy, (C₁-C₆)alkylthio, acylamino, optionally substituted phenyl or heteroaryl, NH₂, or a mono- or di-(C₁-C₆ alkyl) amine;

R₅ is hydrogen or a (C₁-C₆)alkyl group;

provided that at least one of the groups R₃ and R₄ contains a group R₆ as defined above

or a salt, hydrate or solvate thereof.

As used herein, the term "side chain of a naturally occurring amino acid" includes the side chains of alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glycine, histidine, 5-hydroxylysine, 4-hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, alpha-amino adipic acid, alpha-amino-n-butyric acid, 3,4-dihydroxyphenylalanine, homoserine, alpha-methylserine, ornithine, piperolic acid, and thyroxine. The amino acid side chains may be protected; for example the carboxyl groups of aspartic acid, glutamic acid and alpha-amino adipic acid may be esterified (for example as a C₁-C₆ alkyl ester), the amino groups of lysine, ornithine, 5-hydroxylysine, 4-hydroxyproline may be converted to amides (for example as a COC₁-C₆ alkyl amide) or carbamates (for example as a C(=O)OC₁-C₆ alkyl or C(=O)OCH₂Ph carbamate), the hydroxyl groups of 5-hydroxylysine, 4-hydroxyproline, serine, threonine, tyrosine, 3,4-dihydroxyphenylalanine, homoserine, alpha-methylserine and thyroxine may be converted to ethers (for example a C₁-C₆ alkyl or a (C₁-C₆ alkyl)phenyl ether) or esters (for example a C(=O)C₁-C₆ alkyl ester) and the thiol group of cysteine may be converted to thioethers (for example a C₁-C₆ alkyl thioether) or thioesters (for example a C(=O)C₁-C₆ alkyl thioester).

The unqualified term "heterocycl" or "heterocyclic" refers to a 5-7 membered heterocyclic ring containing one or more heteroatoms selected from S, N and O, and optionally fused to a benzene ring, including for example, pyrrolyl, furyl, thienyl, imidazolyl, oxazolyl, thiazolyl, pyrazolyl, pyridinyl, pyrrolidinyl, pyrimidinyl, morpholinyl, piperazinyl, indolyl, benzimidazole, phthalimido, 1,2-dimethyl-3,5-dioxo-1,2,4-triazolidin-4-yl, 3-methyl-2,5-dioxo-1-imidazolidinyl and 3,4,4-trimethyl-2,5-dioxo-1-imidazolidinyl.

As used herein, the term "heteroaryl" refers to a 5- or 6- membered substituted or unsubstituted aromatic heterocycle containing one or more heteroatoms.

Illustrative of such rings are thienyl, furyl, pyrrolyl, imidazolyl, thiazolyl, pyrazolyl, isoxazolyl, isothiazolyl, trizolyl, thiadiazolyl, oxadiazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl and triazinyl.

Unless otherwise specified in the context in which it occurs, the term "substituted" as applied to any moiety herein means substituted with up to four substituents, each of which independently may be C₁-C₆ alkoxy, hydroxy, thio, C₁-C₆ alkylthio, amino, halo (including fluoro, chloro, bromo and iodo), trifluoromethyl, nitro, -COOH, -CONH₂ or -CONH^R_A wherein R^A is a C₁-C₆ alkyl group or the residue of a natural alpha-amino acid.

Salts of the compounds of the invention include physiologically acceptable acid addition salts for example hydrochlorides, hydrobromides, sulphates, methane sulphonates, p-toluenesulphonates, phosphates, acetates, citrates, succinates, lactates, tartrates, fumarates and maleates. Salts may also be formed with bases, for example sodium, potassium, magnesium, and calcium salts.

There are several chiral centres in the compounds according to the invention because of the presence of asymmetric carbon atoms. The presence of several asymmetric carbon atoms gives rise to a number of diastereomers with R or S stereochemistry at each chiral centre. General formula (I), and (unless specified otherwise) all other formulae in this specification are to be understood to include all

such stereoisomers and mixtures (for example racemic mixtures) thereof.

In the compounds of the invention, the preferred stereochemistry is in general as follows:

C atom carrying the R₁ and X groups - S,

C atom carrying the R₂ group - R,

C atom carrying the R₃ group - S,

but mixtures in which the above configurations predominate are also contemplated.

The main invention-characterising feature of the compounds of the invention is the presence of the group R₆ in at least one of the groups R₃ and R₄. Otherwise in the compounds of the invention the definitions of the groups R₁-R₅ are based on groups known from the corresponding positions of known hydroxamic and carboxylic acid-based MMP inhibitors.

Thus, in the compounds of the invention:

R₁ may for example be hydrogen, methyl, ethyl, hydroxyl, allyl, or thiienylmethylsulfanyl. Presently preferred are compounds in which R₁ is hydrogen, hydroxyl or allyl.

R₂ may for example be a C₃-C₈ alkyl, cycloalkyl(C₃-C₆ alkyl), phenyl(C₂-C₆ alkyl), C₂-C₄ alkoxy(C₁-C₃ alkyl)_m or C₂-C₄ alkylsulphanyl(C₁-C₃ alkyl)_m group wherein m is 0 or 1. Examples of particular R₂ groups include isobutyl, n-pentyl, n-hexyl, n-septyl, n-octyl, cyclohexylpropyl, cyclohexylbutyl, cyclohexylpentyl, phenylethyl, phenylpropyl, phenylbutyl, phenylpentyl, propyloxymethyl and propylsulphanyl. Presently preferred are compounds in which R₂ is isobutyl.

(In those cases when R₃ does not comprise a group R₆) examples of suitable R₃ groups include benzyl, iso-butyl and t-butyl. Presently preferred are

compounds in which R_3 is benzyl or t-butyl. Presently most preferred are compounds in which R_3 is t-butyl.

(In those cases when R_4 does not comprise a group R_6) R_4 may for example be C_1-C_6 alkyl, (C_1-C_4) perfluoroalkyl or a group $D-(C_1-C_6$ alkyl) wherein D represents hydroxy, (C_1-C_6) alkoxy, (C_1-C_6) alkylthio, acylamino, optionally substituted phenyl or heteroaryl. Examples of particular R_4 groups include methyl, ethyl, propyl, butyl, hydroxyethyl, hydroxypropyl, 2,2-dimethyl-3-hydroxypropyl, hydroxybutyl, methoxyethyl, ethoxyethyl, methoxypropyl, 2,2-dimethyl-3-methoxypropyl, 2,2-dimethyl-3-ethoxypropyl, 2-ethylthioethyl, 2-acetoxyethyl, N-acetyl-aminoethyl, 3-(2-pyrrolidone)propyl, optionally substituted phenylethyl, phenylpropyl, phenylbutyl and phenylpentyl. Presently preferred are compounds in which R_4 is methyl, t-butyl or benzyl. Presently most preferred are compounds in which R_4 is methyl.

R_5 may for example be hydrogen, methyl or ethyl. Presently preferred are compounds in which R_5 is hydrogen.

Interesting compounds of the invention include:

[4-(N-Hydroxyamino)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycyl-N-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-amide;

[4-(N-Hydroxyamino)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycyl-glycyl-N-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-amide;

[4-(N-Hydroxyamino)-2R-isobutylsuccinyl]-O-[(2-acetamido-2-deoxy- β -D-glucopyranosyl)carboxamidomethyl]-L-tyrosine-N-methylamide;

3S-Hydroxy-4-(N-hydroxyamino)-2R-isobutylsuccinyl-L-phenylalaninyl-glycyl-N-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-amide;

and salts, solvates and hydrates thereof.

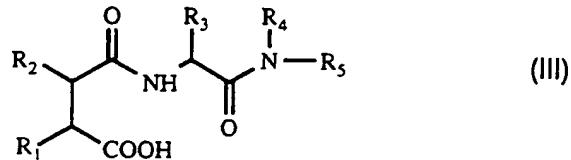
A compound of the invention which is especially preferred for its potency in inhibiting the production of TNF is:

[3S-Allyl-4-(N-hydroxyamino)-2R-isobutylsuccinyl]-N⁴-(2-acetamido-2-deoxy- α -D-glucopyranosyl)-L-glutamine-N-methylamide

and salts, solvates and hydrates thereof.

Compounds according to the present invention in which X is a hydroxamic acid group (-CONHOH) may be prepared from compounds of the invention in which X is a carboxylic acid group (-COOH). That process, which forms another aspect of the invention, comprises:

(a) coupling an acid of general formula (III)



or an activated derivative thereof with hydroxylamine, O-protected hydroxylamine, or a salt thereof, R₂, R₃, R₄, and R₅ being as defined in general formula (I) except that any substituents in R₂, R₃, R₄, and R₅ which are potentially reactive with hydroxylamine, O-protected hydroxylamine or their salts may themselves be protected from such reaction, then removing any protecting groups from the resultant hydroxamic acid moiety and from any protected substituents in R₂, R₃, R₄, and R₅; and

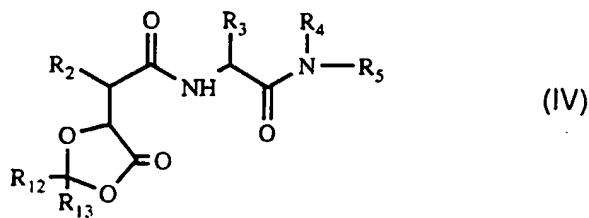
(b) optionally converting a compound of general formula (I) into another compound of general formula (I).

Conversion of (III) to an activated intermediate such as the pentafluorophenyl, hydroxysuccinyl, or hydroxybenzotriazolyl ester may be effected by reaction with the appropriate alcohol in the presence of a dehydrating agent such as dicyclohexyl carbodiimide (DCC), N,N-dimethylaminopropyl-N'-ethyl carbodiimide (EDC), or 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ).

Protecting groups as referred to above are well known per se, for example from the techniques of peptide chemistry. Amino groups are often protectable by benzyloxycarbonyl, tert-butoxycarbonyl or acetyl groups, or in the form of a phthalimido group. Hydroxy groups, for example those hydroxy groups in the group R₆ of the compounds of the invention, may be protected in the above process as readily cleavable ethers such as the tert-butyl or benzyl ether, or as readily cleavable esters such as the acetate. Carboxy groups are often protectable as readily cleavable esters, such as the tert-butyl or benzyl ester.

Compounds of formula (I) above in which the hydroxy groups in the group R₆ are protected, for example as the acetate derivatives, are useful intermediates in the preparation of compounds of the invention.

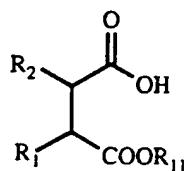
In the special case where R₁ in compound (I) is hydroxy, it too may be protected during the coupling of acid (III) with hydroxylamine. In that case a particularly useful technique may be simultaneous protection of the hydroxy group R₁ and the adjacent carboxyl group as a dioxalone of formula (IV):



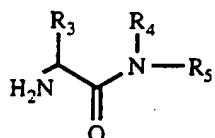
wherein the groups R₁₂ and R₁₃ are derived from a dioxalone forming reagent, and may be, for example, hydrogen, alkyl, phenyl or substituted phenyl. The dioxalone

ring being opened on reaction with hydroxylamine to give the required hydroxamic acid derivative of formula (I).

Compounds according to the present invention in which X is a carboxylic acid group -COOH may be prepared by a process comprising: coupling an acid of formula (V) or an activated derivative thereof with an amine of formula (VI)



(V)

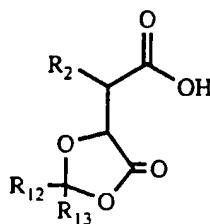


(VI)

wherein R_1 , R_2 , R_3 , R_4 , and R_5 are as defined in general formula (I) except that any substituents in R_1 , R_2 , R_3 , R_4 , and R_5 which are potentially reactive in the coupling reaction may themselves be protected from such reaction, and R_{11} represents a hydroxy protecting group, and subsequently removing the protecting group R_{11} and any protecting groups from R_1 , R_2 , R_3 , R_4 , and R_5 .

Active derivatives of acids (V) include activated esters such as the pentafluorophenyl ester, acid anhydrides and acid halides, eg chlorides. Suitable hydroxy protecting groups may be selected from those known in the art.

In the special case where R_1 in compound (V) is hydroxy, it too may be protected during the coupling of compounds (V) and (VI). In that case a particularly useful technique may be simultaneous protection of the two hydroxy groups as a dioxalane of formula (VII):



(VII)

wherein the groups R_{12} and R_{13} are derived from a dioxalone forming reagent, and may be, for example, hydrogen, alkyl, phenyl or substituted phenyl.

As mentioned above, compounds of formula (I) are useful in human or veterinary medicine since they are active as inhibitors of MMPs, and a further advantage lies in their ability to inhibit the release of tumour necrosis factor (TNF) from cells.

Accordingly in another aspect, this invention concerns:

- (i) a method of management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF in mammals, in particular in humans, which method comprises administering to the mammal an effective amount of a compound as defined with respect to formula (I) above, or a pharmaceutically acceptable salt thereof; and
- (ii) a compound as defined with respect to formula (I) for use in human or veterinary medicine, particularly in the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF; and
- (iii) the use of a compound as defined with respect to formula (I) in the preparation of an agent for the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF.

Diseases or conditions mediated by MMPs include those involving tissue breakdown such as bone resorption, inflammatory diseases, dermatological conditions and tumour invasion by secondary metastases, in particular rheumatoid arthritis, osteoarthritis, periodontitis, gingivitis, corneal ulceration and tumour invasion by secondary metastases. Diseases or conditions mediated by TNF include inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, graft versus host reactions and autoimmune disease.

In a further aspect of the invention there is provided a pharmaceutical or veterinary composition comprising a compound of formula (I) together with a pharmaceutically or veterinarily acceptable excipient or carrier. In view of the oral bioavailability advantages of compounds in accordance with the invention, a further aspect of the invention comprises a pharmaceutical or veterinary composition comprising a compound of formula (I) together with a pharmaceutically or veterinarily acceptable excipient or carrier, characterised in that the composition is adapted for oral administration.

One or more compounds of general formula (I) may be present in the composition together with one or more excipient or carrier.

The compounds with which the invention is concerned may be prepared for administration by any route consistent with their pharmacokinetic properties. The orally administrable compositions may be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions. Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrrolidone; fillers for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricant, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin, hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for

example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

The dosage unit involved in oral administration may contain from about 1 to 250mg, preferably from about 25 to 250mg of a compound of the invention. A suitable daily dose for a mammal may vary widely depending on the condition of the patient. However, a dose of a compound of general formula I of about 0.1 to 300mg/kg body weight, particularly from about 1 to 100mg/kg body weight may be appropriate.

For topical application to the skin, the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations which may be used for the drug are conventional formulations well known in the art, for example as described in standard textbooks of pharmaceutics such as the British Pharmacopoeia.

For topical application to the eye, the drug may be made up into a solution or suspension in a suitable sterile aqueous or non aqueous vehicle. Additives, for instance buffers such as sodium metabisulphite or disodium edeate; preservatives including bactericidal and fungicidal agents such as phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorhexidine, and thickening agents such as hypromellose may also be included.

The dosage for topical administration will of course depend on the size of the area being treated. For the eyes, each dose may typically be in the range from 10 to 100mg of the drug.

The active ingredient may also be administered parenterally in a sterile medium. Depending on the vehicle and concentration used, the drug can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle.

For use in the treatment of rheumatoid arthritis, the drug can be administered by the oral route or by injection intra-articularly into the affected joint. The daily dosage for a 70kg mammal may be in the range 10mgs to 1gram.

The examples which follow illustrate embodiments of the invention but are not intended to limit the scope in any way. The amino acids used in the examples were commercially available or were prepared according to literature procedures.

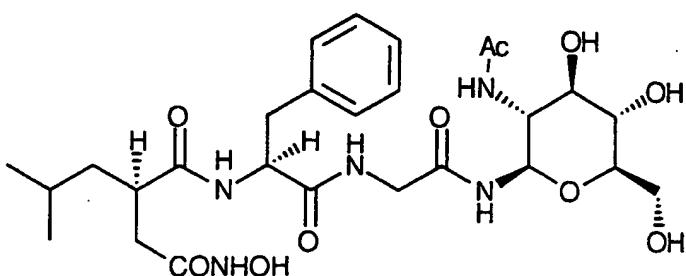
The following abbreviations have been used throughout:

DMF	N,N-Dimethylformamide
EDC	N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride
EEDQ	2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
HOBt	1-Hydroxybenzotriazole
NMM	N-Methylmorpholine
THF	Tetrahydrofuran
TFA	Trifluoroacetic acid
TLC	Thin layer chromatography

¹H and ¹³C NMR spectra were recorded using a Bruker AC 250E spectrometer at 250.1 and 62.9 MHz, respectively. Elemental microanalyses were performed by CHN Analysis Ltd., Alpha House, Countesthorpe Road, South Wigston, Leicester LE8 2PJ, UK.

Example 1

[4-(N-Hydroxyamino)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycyl-N-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-amide.

**Example 1a**

N^{α} -(Benzylloxycarbonyl)-glycyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide

N^{α} -Benzylloxycarbonyl glycine (2.09 g, 10 mmol) and 1-amino-2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranose (3.46 g, 10 mmol) were dissolved in chloroform (100 ml) and stirred during the addition of EEDQ (2.47 g, 10 mmol) in chloroform (5 ml). The reaction mixture was stirred overnight at room temperature then worked up by washing with hydrochloric acid (1M, 2 x 100 ml), sodium carbonate (0.5M, 2 x 100 ml) and saturated brine. The organic layer was dried over magnesium sulphate, filtered and the solvent removed under vacuum to give a white crystalline solid. This material was further purified by recrystallisation from ethyl acetate / methanol (5:1, 180 ml) to yield the title compound (3.32 g, 6.2 mmol, 62%); ^1H NMR: δ ((CD_3)₂SO), 8.40 (1H, d, J = 8.9 Hz), 7.96 (1H, d, J = 9 Hz), 7.44 (1H, t, J = 6.1 Hz), 7.36 (5H, s), 5.20 - 5.07 (2H, m), 5.03 (2H, s), 4.83 (1H, t, J = 9.7 Hz), 4.20 - 3.80 (4H, m), 3.33 (2H, m), 2.00 (3H, s), 1.97 (3H, s), 1.92 (3H, s), and 1.77 (3H, s); ^{13}C NMR δ ((CD_3)₂SO), 172.2, 171.5, 170.7, 170.4, 168.5, 156.4, 136.1, 128.5, 128.1, 80.0, 77.5, 77.0, 76.5, 73.4, 72.7, 63.0, 62.2, 61.2, 52.9, 44.4,

22.9, 20.7 and 20.5.

Example 1b

$\text{N}^{\alpha}-(\text{Benzylloxycarbonyl})\text{-phenylalaninyl-glycyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-}\beta\text{-D-glucopyranosyl)-amide}$

$\text{N}-(\text{Benzylloxycarbonyl})\text{-glycyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-}\beta\text{-D-glucopyranosyl)-amide}$ (3 g, 5.6 mmol) was suspended in methanol and 10% palladium on charcoal (100 mg) added as a slurry in ethyl acetate. Hydrogen gas was passed through the solution for 90 minutes after which time TLC (10% methanol / chloroform) indicated the reaction was complete. The catalyst was removed by filtration then solvent removal gave a white crystalline solid which was used without further purification. This solid was dissolved in DMF (5 ml) and treated with N -benzylloxycarbonyl-phenylalanine pentafluorophenyl ester (2.62 g, 5.6 mmol) overnight at room temperature. Dichloromethane (30 ml) was added to the reaction mixture and the precipitated product filtered off. The solid (1.47 g) was washed with dichloromethane then dried under vacuum. Further product was recovered from the mother liquors by column chromatography (silica gel, 5% methanol / dichloromethane as eluant) followed by crystallisation. The solids were combined to yield 2.37 g (3.46 mmol, 62%) of the desired product; ^1H NMR: δ ((CD_3)₂SO, 323K), 8.27 (1H, d, J = 9.0 Hz), 8.09 (1H, br t), 7.82 (1H, d, J = 9 Hz), 7.40 - 7.14 (11H, m), 5.18 (2H, m), 4.96 (2H, m), 4.85 (1H, t), 4.32 (1H, dt, J = 4.2, 12.4 Hz), 4.20 - 3.80 (4H, m), 3.75 (2H, m), 3.10 (1H, dd, J = 4.2, 14.0 Hz), 2.80 (1H, dd, J = 10.2, 13.8 Hz), 2.00 (3H, s), 1.98 (3H, s), 1.92 (3H, s), and 1.78 (3H, s); ^{13}C NMR: δ ((CD_3)₂SO), 171.4, 169.7, 169.6, 169.2, 169.0, 155.5, 137.9, 136.8, 128.9, 128.0, 127.7, 127.3, 127.1, 125.9, 78.2, 73.0, 72.2, 68.5, 65.1, 61.7, 55.9, 52.1, 41.8, 40.5, 40.1, 39.9, 39.5, 39.2, 38.8, 38.5, 32.3, 22.3, 20.2 and 20.0.

Example 1c

[4-(*tert*-Butoxy)-2*R*-isobutylsuccinyl]-L-phenylalaninyl-glycanyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide.

N-(Benzylloxycarbonyl)-phenylalaninyl-glycanyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide (2.37 g, 3.46 mmol) was suspended in methanol (100 ml) and 10% palladium on charcoal (100 mg) added as a slurry in ethyl acetate. Hydrogen gas was bubbled through the solution for 2 hours after which time the suspended solid had dissolved and tlc (10% methanol / dichloromethane) indicated the reaction was complete. The catalyst was removed by filtration and solvent removed under reduced pressure to leave a white solid. This amine was dissolved in DMF (5 ml), 2*R*-isobutylsuccinic acid-1-pentafluorophenyl-4-*tert*-butyl diester (2.74 g, 6.9 mmol, 2 equivalents) (prepared as described in patent WO 92/13831) was added and the mixture was stirred over the weekend at room temperature. The reaction solvent was removed under reduced pressure, the residual oil taken up in dichloromethane and washed with 1M hydrochloric acid, 0.5M sodium carbonate and saturated brine. Purification away from excess pentafluorophenyl ester was achieved by column chromatography (silica gel, dichloromethane and ethyl acetate) to give the desired product (2.33 g, 3.05 mmol, 88%); ^1H NMR: δ ((CD₃)₂SO), 7.54 (1H, d, J = 8.7 Hz), 7.40 (1H, t, J = 5.7 Hz), 7.28 - 7.12 (6H, m), 6.84 (1H, d, J = 8.9 Hz), 5.32 - 5.17 (2H, m), 5.07 (1H, t, J = 9.7 Hz), 4.81 (1H, m), 4.31 - 3.61 (6H, m), 3.33 (1H, dd, J = 5.5, 14.2 Hz), 2.97 (1H, dd, J = 9.3, 14.2 Hz), 2.68 - 2.35 (3H, m), 2.05 (3H, s), 2.04 (3H, s), 2.02 (3H, s), 2.01 (1H, s), 1.54 - 1.15 (12H, m and s), 0.87 (3H, d, J = 6.3 Hz), and 0.81 (3H, d, J = 6.3 Hz); ^{13}C NMR: δ ((CD₃)₂SO), 175.0, 172.0, 171.9, 171.8, 170.8, 170.4, 170.2, 169.3, 157.3, 128.1, 128.2, 126.4, 80.0, 79.0, 77.5, 77.0, 76.5, 73.0, 72.8, 68.2, 61.8, 55.5, 52.5, 45.3, 41.2, 40.9, 38.0, 36.9, 27.9, 25.4, 23.0, 22.7, 22.1, and 20.5.

Example 1d

[4-(Benzyl oxyamino)-2*R*-isobutylsuccinyl]-L-phenylalaninyl-glycanyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide.

[4-(tert-Butoxy)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide (2.23 g, 2.9 mmol) in dichloromethane (10 ml) was treated with TFA (5 ml) at room temperature. After stirring overnight at room temperature the solvents were removed under vacuum and the residual oil azeotroped with chloroform in an attempt to remove excess TFA. 1 H NMR analysis indicated complete conversion to the acid and the material was used without further purification. The acid was dissolved in DMF cooled to 0°, then HOBT (1.33g, 8.7 mmol), NMM (0.3g, 2.9 mmol), O-benzylhydroxylamine (1.07g, 8.7 mmol) and finally EDC (0.83 g, 4.3 mmol) added. The reaction mixture was stirred for six hours, a further portion (0.5 g) of EDC added and the reaction allowed to stir overnight. The reaction was worked up by removing DMF under reduced pressure, redissolving the residue in dichloromethane (200 ml) then washing with 1M hydrochloric acid (2 x 100 ml), 0.5M sodium carbonate (2 x 100 ml) and saturated brine. The organic layer was dried over magnesium sulphate, filtered and the solvent removed to leave crude product which was purified by column chromatography (silica gel, 5% methanol / dichloromethane) to give the title compound (1.78 g, 2.19 mmol, 76%); 1 H NMR: δ ((CD₃)₂SO, 353K), 10.62 (1H, br s), 7.97 (1H, br d, J = 8.4 Hz), 7.79 (2H, br m), 7.63 (1H, br d, J = 8.0 Hz), 7.37 (5H, m), 7.23 (5H, m), 5.20 (2H, m), 4.85 (1H, t, J = 9.6 Hz), 4.53 (1H, dt, J = 5.3, 8.4 Hz), 4.19 - 3.70 (6H, m), 3.15 (1H, dd, J = 5.2, 14.1 Hz), 2.92 (1H, dd, J = 9.0, 14.1 Hz), 2.66 (1H, m), 2.17 (1H, dd, J = 6.8, 15.0 Hz), 2.02 (1H, m), 1.99 (3H, s), 1.98 (3H, s), 1.93 (3H, s), 1.80 (3H, s), 1.42 (2H, m), 1.09 (1H, m), 0.83 (3H, d, J = 6.1 Hz), and 0.79 (3H, d, J = 6.1 Hz).

Example 1e

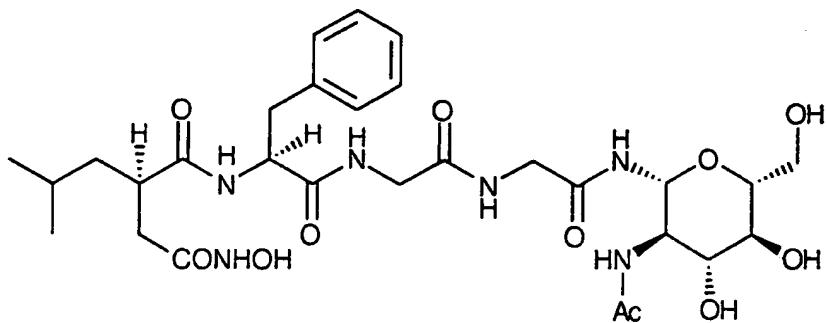
[4-(N-Hydroxyamino)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycyl-N-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-amide

[4-(Benzylamino)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide (1.59 g, 1.96 mmol) was dissolved in methanol and sodium methoxide (0.2M in methanol, 0.5

ml) added. After two hours at room temperature tlc (10% methanol / dichloromethane) indicated complete conversion and the reaction was neutralised with an ion exchange resin (Amberlite 120 / H⁺). The resin was filtered off, the solvent removed under reduced pressure to leave an amorphous solid. This material was then hydrogenated for 30 minutes in methanol (20 ml) using 10% palladium on charcoal (100 mg) as catalyst to give the title hydroxamic acid as an amorphous solid which was further purified by trituration with ethyl acetate (1.02 g, 1.72 mmol, 87%); Analysis: C₂₇H₄₁N₅O₁₀.1.7H₂O requires C, 51.78%, H, 7.15%, N, 11.18%; Found C, 51.64%, H, 6.77%, N, 10.85%; ν_{max} (KBr) 3512, 3302, 1654, 1541 cm⁻¹; ¹H NMR: δ ((CD₃)₂SO, 323K), 10.30 (1H, br s), 7.98 (2H, br m), 7.23 (2H, br m), 7.25 - 7.14 (5H, m), 4.81 (1H, br dd), 4.53 (1H, m), 3.76 - 3.36 (6H, m), 3.21 - 3.08 (3H, m), 2.88 (1H, dd, J = 9.6, 13.9 Hz), 2.63 (1H, br m), 2.08 - 1.90 (2H, br m), 1.86 (3H, s), 1.44 - 1.29 and 1.10 - 0.98 (3H, m), 0.79 (3H, d, J = 6.3 Hz) and 0.75 (3H, d, J = 6.3 Hz); ¹³C NMR δ ((CD₃)₂SO, 323 K), 173.8, 171.2, 170.6, 168.9, 167.4, 138.1, 128.9, 127.7, 125.8, 79.1, 78.4, 74.0, 70.5, 60.8, 54.4, 53.6, 42.2, 40.5, 36.7, 35.5, 24.9, 22.9, 22.5, and 21.6.

Example 2

[4-(N-Hydroxyamino)-2R-isobutylsuccinyl]-L-phenylalanine-glycyl-glycyl-N-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-amide



Example 2a

N-(Benzylloxycarbonyl)-glycyl-glycyl-N-(2-acetamido-3,4,6,-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide

N-(Benzylloxycarbonyl)-glycyl-glycine (3.00 g, 8.67 mmol) and 1-amino-2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranose (2.31 g, 8.67 mmol) were dissolved in DMF (100 ml) and stirred during addition of EEDQ (2.14 g, 8.67 mmol). The reaction mixture was stirred overnight at room temperature. DMF was removed under reduced pressure and the residue partitioned between ethyl acetate and 1M hydrochloric acid. The organic layer was separated and washed with 1M hydrochloric acid, saturated sodium bicarbonate (x 3) and brine. The solution was dried over magnesium sulphate, filtered and the solvent removed under vacuum to give the title compound as a white crystalline solid (2.15 g, 3.62 mmol, 42%). ^1H NMR: δ ((CD₃)₂SO), 8.34 (1H, d, J = 8.7 Hz), 8.12, (1H, br s), 7.99 (1H, d, J = 9.0 Hz), 7.37 (1H, br s), 7.36 (5H, s), 5.14 (2H, t, J = 9.7 Hz), 5.05 (2H, s), 4.83 (1H, t, J = 9.8 Hz), 4.18 (1H, dd, J = 12.5, 4.2 Hz), 3.99 - 3.73 (3H, m), 3.70 (2H, d, J = 5.8), 1.99 (3H, s), 1.97 (3H, s), 1.92 (3H, s) and 1.77 (3H, s); ^{13}C NMR: δ ((CD₃)₂SO), 170.2, 170.0, 169.5, 169.3, 156.5, 137.0, 128.3, 127.7, 78.3, 72.9, 72.2, 68.5, 65.5, 61.8, 52.0, 43.6, 41.9, 22.5, 20.5 and 20.4.

Example 2b

N-(Benzylloxycarbonyl)-phenylalaninyl-glycyl-glycyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide

N-(Benzylloxycarbonyl)-glycyl-glycyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide (2.05 g, 3.20 mmol) was dissolved in a 1:1 mixture of methanol / ethyl acetate (30 ml) and 10% palladium on charcoal (50 mg) added. Hydrogen gas was passed through the solution for 150 minutes. The catalyst was removed by filtration. Evaporation of solvent under reduced pressure gave a white solid which was used without further purification. The solid was dissolved in DMF (25 ml) and treated with N-benzylloxycarbonyl-phenylalanine pentafluorophenyl ester (1.57 g, 3.38 mmol). The reaction was left to stand at room temperature

overnight. DMF was removed under reduced pressure and the residue partitioned between ethyl acetate and 1M hydrochloric acid. The organic layer was separated and washed with 1M hydrochloric acid (x3), saturated sodium bicarbonate (x3) and brine. The solution was dried over magnesium sulphate. Filtration and evaporation of solvent under reduced pressure, followed by recrystallisation from ethyl acetate/hexane gave the desired product as a white crystalline solid (2.30 g, 2.74 mmol, 86%); ^1H NMR: δ ((CD₃)₂SO), 8.36 (1H, d, J = 9.1 Hz), 8.27, (1H, br s), 8.06 - 7.95 (2H, m), 7.55 (1H, d, J = 8.6 Hz), 7.33 - 7.21 (10H, m), 5.14 (2H, t, J = 10.0 Hz), 4.94 (2H, d, J = 3.7 Hz), 4.83 (1H, t, J = 9.7 Hz), 4.31 (1H, m), 4.17 (1H, dd, J = 12.6, 4.1 Hz), 3.97 - 3.69 (7H, m), 3.07 (1H, dd, J = 13.8, 3.5 Hz), 2.78 (1H, d, J = 13.1 Hz), 1.98 (3H, s), 1.96 (3H, s), 1.91 (3H, s) and 1.77 (3H, s).

Example 2c

[4-(*tert*-Butoxy)-2*R*-isobutylsuccinyl]-L-phenylalaninyl-glycanyl-glycanyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide

N-(Benzylxycarbonyl)-phenylalaninyl-glycanyl-glycanyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide (1.95 g, 2.63 mmol) was dissolved in a 1:1 mixture of methanol/ethyl acetate (20 ml) and 10% palladium on charcoal (50 mg) added. Hydrogen gas was bubbled through the solution for 90 minutes. The catalyst was removed by filtration and solvent removed under reduced pressure to leave a white solid. The amine was dissolved in DMF (25 ml), 2*R*-isobutylsuccinic acid-1-pentafluorophenyl-4-*tert*-butyl diester (1.18 g, 2.98 mmol) (prepared as described in patent WO 92/13831) was added and the reaction was stirred at room temperature for 48 hours. The reaction solvent was removed under reduced pressure. The residue was partitioned between ethyl acetate and 1M hydrochloric acid. The organic layer was separated and washed with 1M hydrochloric acid (x2), saturated sodium bicarbonate (x3) and brine before drying over magnesium sulphate, filtration and removal of solvent to leave a colourless oil. Recrystallisation from ethyl acetate / hexane gave the desired product as a white crystalline solid (1.54 g, 1.88 mmol, 71%); ^1H NMR: δ (CD₃OD), 7.23 (5H, m), 5.19

(2H, m), 4.96 (1H, t, J = 9.8 Hz), 4.46 (1H, dd, J = 8.5, 6.9 Hz), 4.22 (1H, dd, J = 12.5, 4.4 Hz), 4.10 - 3.64 (7H, m), 3.18 (1H, dd, J = 13.9, 6.8 Hz), 3.00 (1H, dd, J = 13.8, 8.7 Hz), 2.67 (1H, m), 2.37 (1H, dd, J = 16.0, 8.4 Hz), 2.21 (1H, dd, J = 16.1, 6.1 Hz), 1.96 (3H, s), 1.95 (3H, s), 1.95 (3H, s), 1.87 (3H, s), 1.38 (11H, m and s), 1.11 (1H, m), 0.84 (3H, d, J = 6.3 Hz) and 0.79 (3H, d, J = 6.2 Hz); ^{13}C NMR: δ (CD₃OD), 178.0, 174.3, 174.0, 173.2, 172.2, 172.1, 171.7, 171.3, 138.6, 130.3, 129.5, 127.8, 81.0, 79.9, 74.6, 74.5, 69.9, 63.2, 56.9, 53.9, 43.8, 43.5, 42.6, 41.9, 39.2, 37.9, 28.3, 26.6, 23.3, 22.8, 22.6 and 20.6.

Example 2d

[4-(Benzylxyamino)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycyl-glycyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide

[4-(*tert*-Butoxy)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycyl-glycyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide (650 mg, 0.79 mmol) was dissolved in trifluoroacetic acid (20 ml) and allowed to stand at room temperature for 4 hours. TFA was removed under reduced pressure and the product was crystallised from dichloromethane / diethyl ether to yield the required product (365 mg, 0.48 mmol, 61%). The acid (150 mg, 0.20 mmol) was dissolved in DMF (15 ml) and treated with HOBr (25 mg, 0.20 mmol), O-benzylhydroxylamine (25 mg, 0.20 mmol) and finally EDC (39 mg, 0.20 mmol). The reaction was stirred at room temperature overnight. DMF was removed under reduced pressure. The residue was partitioned between ethyl acetate and 1M hydrochloric acid. The organic layer was separated and washed with 1M hydrochloric acid (x 2), saturated sodium bicarbonate (x 3) and brine, then dried over magnesium sulphate, filtered and the solvent evaporated to leave a colourless oil. Crystallisation from ethyl acetate/hexane provided the title compound as a white solid (123 mg, 0.14 mmol, 71%); ^1H NMR: δ (CD₃OD), 7.42 - 7.17 (10H, m), 5.26 - 5.18 (2H, m), 4.85 (1H, t, J = 9.5 Hz), 4.45 (1H, m), 4.28 - 3.70 (8H, m), 3.15 (1H, dd, J = 5.0, 14.1 Hz), 3.02 (1H, dd, J = 9.0, 14.2 Hz), 2.68 (1H, m), 2.21 (1H, dd, J = 6.9, 14.8 Hz), 2.03 (1H, m), 2.02 (3H, s), 2.00 (3H, s), 1.98 (3H, s), 1.92 (3H, s), 1.35 (2H, m), 1.06 (1H, m), and

0.78 (6H, t, J = 6.7 Hz).

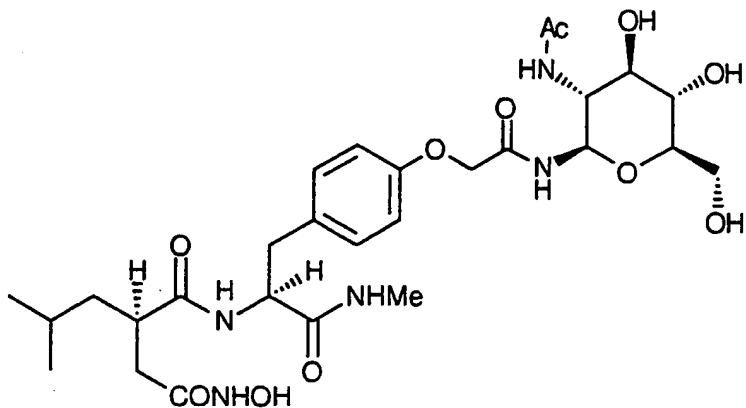
Example 2e

[4-(N-Hydroxyamino)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycyl-glycyl-N-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-amide

[4-(Benzylxyamino)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycylglycyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide (110 mg, 0.13 mmol) was dissolved in methanol (10 ml) and sodium methoxide (8 mg, 0.15 mmol in methanol, 0.5 ml) added. After 3 hours the reaction was neutralised with ion-exchange resin (amberlite 120/H⁺). The resin was filtered off and washed with methanol. The combined filtrate was treated with 10% palladium on charcoal (50 mg) and stirred under an atmosphere of hydrogen for 90 minutes. Filtration followed by evaporation of solvent gave a white solid. Recrystallisation from methanol/diethyl ether gave the title compound (48 mg, 0.07 mmol, 57%). m.p. 187°C; ¹H NMR: δ (CD₃OD), 7.25 - 7.15 (5H, m), 4.89 (1H, m), 4.44 (1H, dd, J = 9.2, 6.0 Hz), 4.01-3.43 (11H, m), 3.24 (1H, m), 3.04 (1H, dd, J = 13.8, 9.5 Hz), 2.66 (1H, m), 2.24 (1H, dd, J = 14.8, 5.9 Hz), 2.08 (1H, dd, J = 14.5, 5.9 Hz), 1.95 (3H, s), 1.36 (2H, m), 1.07 (1H, m), and 0.77 (6H, t, J = 6.7 Hz); ¹³C NMR: δ (CD₃OD), 178.0, 174.7, 174.2, 172.4, 170.8, 138.8, 130.2, 129.5, 126.7, 80.62, 79.7, 77.3, 71.9, 62.6, 56.9, 55.9, 43.9, 43.6, 42.5, 37.6, 26.6, 23.5, 22.9 and 22.3.

Example 3

[4-(N-Hydroxyamino)-2R-isobutylsuccinyl]-O-[(2-acetamido-2-deoxy- β -D-glucopyranosyl)carboxamidomethyl]-L-tyrosine-N-methylamide



Example 3a

[4-*tert*-Butoxy-2R-isobutylsuccinyl]-O-[(2-acetamido-2-deoxy- β -D-glucopyranosyl)carboxamidomethyl]-L-tyrosine-N-methylamide

[4-*tert*-Butoxy-2R-isobutylsuccinyl]-O-carboxymethyl-L-tyrosine-N-methylamide (prepared as described in patent WO 92/13831, 900 mg, 1.94 mmol), 1-amino-2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranose (671 mg, 1.94 mmol) and EEDQ (478 mg, 1.94 mmol) were dissolved in dichloromethane (20 ml). The reaction mixture was stirred overnight at room temperature resulting in formation of a gelatinous precipitate. Solvent was removed under reduced pressure and the residue partitioned between ethyl acetate (50 ml) and 1M hydrochloric acid (50 ml). The organic layer was separated, washed with 1M hydrochloric acid (2 x 50 ml) and brine (50 ml). On standing for 10 minutes a thick white precipitate formed which was collected by filtration, washed with diethyl ether and dried under vacuum to yield the title compound (925 mg, 1.17 mmol, 60%); 1 H NMR: δ ((CD₃)₂SO), 8.66 (1H, d, J = 8.9 Hz), 8.10 (1H, d, J = 8.2 Hz), 8.04 (1H, d, J = 8.9 Hz), 7.81 (1H, m), 7.11 (2H, d, J = 8.4 Hz), 6.79 (2H, d, J = 8.5 Hz), 5.27 - 5.13 (2H, m), 4.84 (1H, t, J = 9.7 Hz), 4.44 (2H, s), 4.35 (1H, dd, J = 14.7, 8.3 Hz), 4.19 (1H, dd, J

= 12.6, 4.3 Hz), 4.02 - 3.86 (3H, m), 2.88 (1H, dd, J = 13.8, 5.6 Hz), 2.71 (1H, dd, J = 13.8, 8.7 Hz), 2.60 (1H, m), 2.52 (1H, d, J = 7.4 Hz), 2.26 (1H, dd, J = 15.7, 7.3 Hz), 2.09 (1H, dd, J = 15.6, 7.2 Hz), 1.99 (3H, s), 1.97 (3H, s), 1.92 (3H, s), 1.71 (3H, s), 1.35 (11H, s and m), 0.82 (3H, d, J = 6.1 Hz), and 0.75 (3H, d, J = 6.0 Hz); ^{13}C NMR: δ ((CD₃)₂SO), 173.6, 171.3, 170.8, 170.0, 169.5, 169.3, 168.5, 130.0, 114.4, 79.7, 78.2, 72.9, 72.4, 68.8, 62.0, 54.2, 52.2, 38.0, 36.7, 27.7, 25.4, 25.1, 22.9, 22.5, 22.2, 20.5 and 20.2.

Example 3b

[4-Hydroxy-2R-isobutylsuccinyl]-O-[(2-acetamido-2-deoxy- β -D-glucopyranosyl)carboxamidomethyl]-L-tyrosine-N-methylamide

[4-*tert*-Butoxy-2R-isobutylsuccinyl]-O-[(2-acetamido-2-deoxy- β -D-glucopyranosyl)carboxamidomethyl]-L-tyrosine-N-methylamide (250 mg, 0.32 mmol) was dissolved in a mixture of TFA and dichloromethane (1:1, 10 ml) and allowed to stand at room temperature overnight. Solvent was removed under reduced pressure. Addition of diethyl ether (30 ml) gave a white solid which was collected by filtration, washed with diethyl ether (2 x 30 ml) and dried under vacuum to give the title compound (230 mg, 3.0 mmol, 100%), ^1H NMR: δ ((CD₃)₂SO), 8.63 (1H, d, J = 8.8 Hz), 8.03 - 7.98 (2H, m), 7.71 (1H, m), 7.11 (2H, d, J = 8.5 Hz), 6.80 (2H, d, J = 8.3 Hz), 5.24 (1H, t, J = 9.2 Hz), 5.18 (1H, t, J = 10.2 Hz), 4.45 (2H, s), 4.35 (1H, m), 4.19 (1H, dd, J = 12.6, 4.3 Hz), 4.03 - 3.86 (3H, m), 2.89 (1H, dd, J = 13.8, 5.7 Hz), 2.74 (1H, dd, J = 13.7, 8.5 Hz), 2.65 (1H, m), 2.54 (3H, d, J = 4.3 Hz), 2.30 (1H, dd, J = 16.0, 7.5 Hz), 2.14 (1H, dd, J = 16.1, 6.7 Hz), 1.99 (3H, s), 1.97 (3H, s), 1.92 (3H, s), 1.72 (3H, s), 1.34 (2H, m), 1.06 (1H, m), 0.82 (3H, d, J = 6.0 Hz) and 0.76 (3H, d, J = 6.0 Hz); ^{13}C NMR: δ ((CD₃)₂SO), 173.8, 173.1, 171.2, 170.0, 169.5, 169.3, 168.5, 156.2, 130.8, 130.0, 114.4, 78.2, 72.9, 72.4, 68.7, 66.9, 61.9, 54.1, 52.2, 37.0, 36.6, 25.5, 25.1, 22.9, 22.5, 20.5 and 20.3.

Example 3c

[4-(N-Benzylamino)-2R-isobutylsuccinyl]-O-[(2-acetamido-2-deoxy- β -D-glucopyranosyl)carboxamidomethyl]-L-tyrosine-N-methylamide

[4-Hydroxy-2R-isobutylsuccinyl]-O-[(2-acetamido-2-deoxy- β -D-glucopyranosyl)carboxamidomethyl]-L-tyrosine-N-methylamide (950 mg, 1.29 mmol) was taken up in DMF (15 ml) and treated with HOBt (174 mg, 1.29 mmol) and EDC (742 mg, 3.87 mmol). After stirring at room temperature for 30 minutes the reaction was treated with O-benzyl hydroxylamine (476 mg, 3.87 mmol) and allowed to stir overnight. The reaction solvent was removed under reduced pressure to leave a colourless gum. Addition of 1M hydrochloric acid (20 ml) and diethyl ether (20 ml) gave a white precipitate. The solid was collected by filtration, washed with distilled water (3 x 20 ml) and diethyl ether (3 x 20 ml) before drying under vacuum to provide the title compound (810 mg, 0.96 mmol, 75%); 1 H NMR: δ ((CD₃)₂SO), 8.51 (1H, br m), 7.91 (2H, br m), 7.73 (1H, br m), 7.37 (5H, s), 7.11 (2H, d, J = 8.4 Hz), 6.79 (2H, d, J = 8.0 Hz), 5.26 - 5.14 (2H, m), 4.84 (1H, t, J = 9.6 Hz), 4.77 (2H, s), 4.43 (2H, s), 4.37 (1H, m), 4.17 (1H, dd, J = 12.4, 4.1 Hz), 4.00 - 3.84 (3H, m), 2.99 - 2.91 (1H, m), 2.81 - 2.75 (1H, m), 2.64 (1H, m), 2.57 (3H, d, J = 4.2 Hz), 2.00 (2H, m), 1.99 (3H, s), 1.97 (3H, s), 1.93 (3H, s), 1.73 (3H, s), 1.33 (2H, m), 1.02 (1H, m), 0.79 (3H, d, J = 6.0 Hz) and 0.74 (3H, d, J = 5.9 Hz).

Example 3d

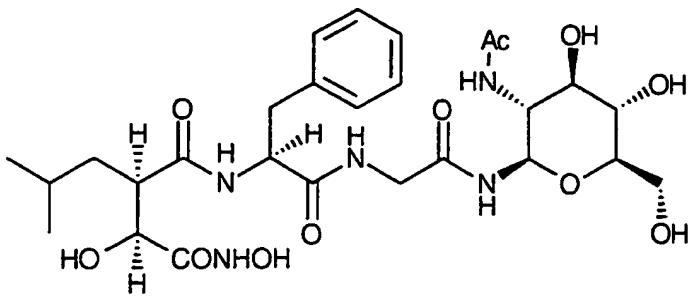
[4-(N-Hydroxyamino)-2R-isobutylsuccinyl]-O-[(2-acetamido-2-deoxy- β -D-glucopyranosyl)carboxamidomethyl]-L-tyrosine-N-methylamide

[4-(N-Benzylamino)-2R-isobutylsuccinyl]-O-[(2-acetamido-2-deoxy- β -D-glucopyranosyl)carboxamidomethyl]-L-tyrosine-N-methylamide (780 mg, 0.93 mmol) was suspended in methanol (25 ml) and sodium methoxide (0.93 mmol) in methanol (5 ml) added. The reaction was allowed to stir at room temperature overnight. The reaction was neutralised with ion exchange resin (Amberlite 120/H⁺). The solution/suspension was decanted from the resin. The reacton

mixture was treated with 10% palladium on charcoal (100 mg) and stirred under an atmosphere of hydrogen for 60 minutes. The catalyst was removed by filtration and solvent was removed under reduced pressure to give a white solid. Recrystallisation from methanol/diisopropyl ether gave the title compound (432 mg, 0.69 mmol, 74%). m.p. 222.6°C; Analysis: $C_{28}H_{43}N_3O_{11}.1.3H_2O$ requires C, 51.81%, H, 7.08%, N, 10.79%; Found C, 51.78%, H, 7.05%, N, 10.77%; 1H NMR: δ ((CD₃)₂SO), 10.40 (1H, s), 8.76 (1H, s), 8.44 (1H, d, J = 8.3 Hz), 8.00 (1H, d, J = 8.2 Hz), 7.93 - 7.85 (2H, m), 7.12 (2H, d, J = 8.4 Hz), 6.82 (2H, d, J = 8.5 Hz), 4.78 (1H, t, J = 9.0 Hz), 4.47 - 4.32 (3H, m), 3.68 - 3.34 (6H, m), 3.14 (2H, s), 2.90 (1H, dd, J = 14.0, 5.1 Hz), 2.74 (1H, dd, J = 13.5, 9.5 Hz), 2.56 (3H, d, J = 4.3 Hz), 2.55 (1H, m), 2.05 (1H, dd, J = 14.3, 6.9 Hz), 1.90 (1H, dd, J = 14.3, 7.6 Hz), 1.76 (3H, s), 1.27 (2H, m), 0.96 (1H, m), 0.78 (3H, d, J = 6.2 Hz) and 0.73 (3H, d, J = 6.4 Hz); ^{13}C NMR: δ ((CD₃)₂SO), 173.6, 171.4, 170.7, 168.5, 167.7, 156.1, 131.0, 130.0, 114.3, 79.2, 78.7, 73.9, 70.5, 66.8, 60.8, 54.3, 54.2, 40.6, 36.4, 35.7, 25.6, 25.1, 23.2, 22.7 and 21.9.

Example 4

[3S-Hydroxy-4-(N-hydroxyamino)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycyl-N-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-amide.



Example 4a

[2R-(2,2-Dimethyl-1,3-dioxalon-5S-yl)-4-methylpentanoyl]-L-phenylalaninyl-

glycanyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide.

N-(Benzylloxycarbonyl)-phenylalaninyl-glycanyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide (Example 1b, 1.15 g, 1.68 mmol) was suspended in methanol (30 ml) and treated with 10% palladium on charcoal (100 mg). The reaction mixture was stirred under an atmosphere of hydrogen for 60 minutes. The catalyst was removed by filtration and solvent evaporated to give a white solid. The amine was dissolved in DMF (15 ml) and treated with 2R-(2,2-dimethyl-4-oxo-1,3-dioxolan-5S-yl)-4-methylpentanoic acid pentafluorophenyl ester (1.33g, 2.26 mmol) (prepared as described in patent WO 94/02447). The reaction mixture was left standing at room temperature overnight. DMF was removed under reduced pressure to leave a colourless oil which was partitioned between ethyl acetate and saturated sodium bicarbonate. The organic layer was separated and washed with saturated sodium bicarbonate (x2) and brine before drying over magnesium sulphate. Filtration and removal of solvent and recrystallisation from ethyl acetate and hexane gave the title compound (825 mg, 1.08 mmol, 64%); 1 H NMR: δ (CD₃OD), 7.22 (5H, s), 5.26 - 5.19 (2H, m), 4.98 (1H, t, J = 10.0 Hz), 4.57 (1H, dd, J = 9.3, 5.4 Hz), 4.51 (1H, d, J = 8.1 Hz), 4.24 (1H, dd, J = 12.4, 4.3 Hz), 4.06 - 3.75 (3H, m), 3.22 (1H, m), 3.06-3.01 (1H, m), 2.70 (1H, m), 1.98 (3H, s), 1.97 (3H, s), 1.95 (3H, s), 1.89 (3H, s), 1.59 (2H, m), 1.49 (3H, s), 1.45 (3H, s), 1.20 (1H, m), 0.84 (3H, d, J = 6.6 Hz) and 0.80 (3H, d, J = 6.5 Hz).

Example 4b

3S,4-Dihydroxy-2R-isobutylsuccinyl-L-phenylalaninyl-glycanyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide.

[2R-(2,2-Dimethyl-1,3-dioxolan-5S-yl)-4-methylpentanoyl]-L-phenylalaninyl-glycanyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide (300 mg, 0.39 mmol) was dissolved in a mixture of TFA / dichloromethane (1:1, 20 ml). The reaction mixture was allowed to stand at room temperature overnight. Solvent and excess TFA were removed under reduced pressure. Addition of

diethyl ether (50 ml) gave a white solid which was collected by filtration. The solid was washed with diethyl ether and dried under vacuum to yield the title compound (265 mg, 0.37 mmol, 94%); ^1H NMR: δ ((CD₃)₂SO), 8.33 (1H, d, J = 9.3 Hz), 8.04 - 9.94 (2H, m), 7.23 (5H, s), 5.21 - 5.10 (2H, m), 4.83 (1H, t, J = 9.7 Hz), 4.52 (1H, m), 3.48 (1H, br s), 4.20 (1H, m), 3.98 - 3.68 (5H, m), 3.15 - 2.90 (2H, m), 2.58 (1H, m), 1.99 (3H, s), 1.97 (3H, s), 1.92 (3H, s) 1.77 (3H, s), 1.48 (1H, m), 1.25 (1H, m), 1.06 (1H, m), 0.79 (3H, d, J = 5.7 Hz) and 0.75 (3H, d, J = 6.4 Hz).

Example 4c

[3S-Hydroxy-4-(N-benzyloxyamino)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycanyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide.

[3S,4-Dihydroxy-2R-isobutylsuccinyl]-2-phenylalaninyl-glycanyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide (250 mg, 0.35 mmol) was dissolved in a mixture of THF/water (1:1, 20 ml). The solution was treated with O-benzylhydroxylamine hydrochloride (88 mg, 0.55 mmol) and EDC (140 mg, 0.73 mmol). The reaction was stirred at room temperature overnight. THF was removed under reduced pressure. A white precipitate formed which was collected by filtration, washed with water (50ml) and dried under vacuum to yield the title compound (195 mg, 0.24 mmol, 67%); ^1H NMR: δ ((CD₃)₂SO), 8.33 (1H, d, J = 9.0 Hz), 8.17 (1H, d, J = 6.3 Hz), 7.99 - 7.90 (2H, m), 7.39 - 7.36 (5H, m), 7.24 - 7.18 (6H, m), 5.67 (1H, d, J = 6.2 Hz), 5.20 - 5.10 (2H, m), 4.84 (1H, t, J = 9.8 Hz), 4.76 (2H, s), 4.53 (1H, m), 4.16 (1H, dd, J = 12.2, 4.4 Hz), 3.97 - 3.82 (3H, m), 3.70 (2H, t, J = 6.0 Hz), 3.11 (1H, dd, J = 14.6, 5.3 Hz), 2.91 (1H, dd, J = 14.1, 9.0 Hz), 2.54 (1H, m), 1.98 (3H, s), 1.97 (3H, s), 1.92 (3H, s), 1.78 (3H, s), 1.43 (1H, m), 1.35 (1H, m), 0.93 (1H, m) and 0.74 (6H, dd, J = 4.5 Hz).

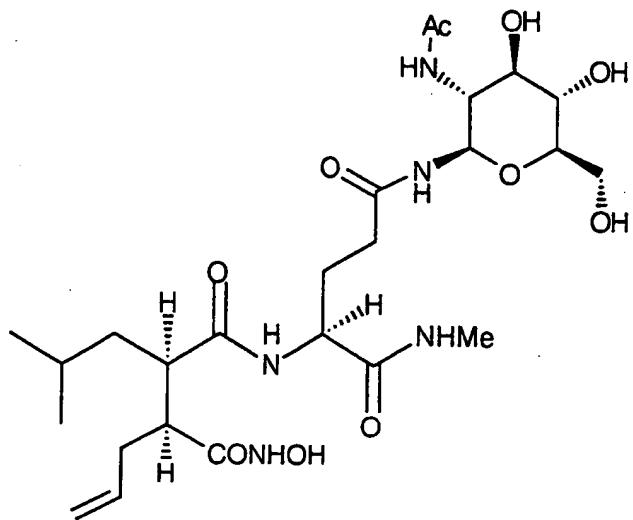
Example 4d

[3S-Hydroxy-4-(N-hydroxyamino)-2R-isobutyl-succinyl]-L-phenylalaninyl-glycanyl-N-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-amide.

A suspension of [3S-hydroxy-4-(N-benzyloxy-amino)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycanyl-N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-amide (160 mg, 0.19 mmol) in methanol (15 ml) was treated with a solution of sodium methoxide (5 mg, 0.22 mmol) in methanol (5 ml). The reaction was neutralised with ion exchange resin (Amberlite 120/H⁺) and the supernatent was decanted. The reaction mixture was treated with 10% palladium on charcoal (50 mg) and stirred under an atmosphere of hydrogen for 60 minutes. The catalyst was removed by filtration and solvent removed under reduced pressure to leave a white solid. Recrystallisation from methanol/diisopropyl ether gave the title compound (75 mg, 0.12 mmol, 63%); Mpt 200.5°C; ¹H NMR: δ ((CD₃)₂SO), 10.40 (1H, s), 8.27 (1H, m), 7.99 - 7.89 (4H, m), 7.22 (5H, m), 4.76 (1H, t, J = 9.1 Hz), 4.52 (1H, m), 3.86 (1H, d, J = 6.8 Hz), 3.75 - 3.32 (6H, m), 3.12 (3H, m), 2.94 (1H, m), 2.50 (1H, m), 1.66 - 1.55 (1H, m), 1.32 - 1.15 (1H, m), 1.04 - 0.90 (1H, m), 0.74 (3H, d, J = 6.2 Hz) and 0.73 (3H, d, J = 6.4 Hz); ¹³C NMR: δ ((CD₃)₂SO), 172.5, 171.3, 170.7, 169.2, 168.7, 138.3, 129.0, 127.9, 126.0, 79.3, 78.6, 74.0, 71.0, 70.4, 60.8, 54.3, 53.8, 42.4, 37.4, 36.7, 24.9, 23.5, 22.7 and 21.5.

Example 5

[3S-Allyl-4-(N-hydroxyamino)-2R-isobutylsuccinyl]-N⁴-(2-acetamido-2-deoxy- α -D-glucopyranosyl)-L-glutamine-N-methylamide



Example 5a

Na^{α} -tert-Butyloxycarbonyl-N⁴-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- α -D-glucopyranosyl)-L-glutamine-N-methylamide

Prepared by methods analogous to those described in Example 1a, starting from Na^{α} -tert-butyloxycarbonyl-L-glutamic acid-N-methylamide (2.80 g, 10.75 mmol) and 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- α -D-glucopyranosylamine (10.75 mmol). Yield: 3.95 g (64%). ¹H-NMR; δ ((CD₃)₂SO), 8.47 (1H, d, J = 9.2 Hz), 7.80 (1H, d, J = 9.0 Hz), 7.70 (1H, br m), 6.82 (1H, br d), 5.18 - 5.05 (2H, m), 4.80 (1H, t, J = 9.8 Hz), 4.21 - 4.14 (1H, m), 3.96 - 3.67 (4H, m), 2.57 (3H, d, J = 4.2 Hz), 2.18 - 2.07 (2H, m), 1.99 (3H, s), 1.96 (3H, s), 1.90 (3H, s), 1.73 (3H, s), 1.73 - 1.62 (2H, m), 1.37 (9H, s); ¹³C-NMR; δ ((CD₃)₂SO), 172.2, 172.0, 169.9, 169.4, 169.2, 78.1, 73.4, 72.3, 68.7, 62.0, 53.9, 52.3, 31.9, 28.2, 27.9, 25.5, 22.5, 20.5 and 20.3.

Example 5b

[3S-Allyl-4-*tert*-butoxy-2R-isobutylsuccinyl]-N⁴-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- α -D-glucopyranosyl)-L-glutamine-N-methylamide

Prepared by methods analogous to those described in Example 1c, starting from 3R,S-allyl-2R-isobutylsuccinic acid-1-pentafluorophenyl-4-*tert*-butyl diester (3:1, RS:RR) (prepared as described in patent PCT/GB94/00495, 4.3 g, 10.1 mmol) and N α -*tert*-butyloxycarbonyl-N⁴-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- α -D-glucopyranosyl)-L-glutamine-N-methylamide (5.9 g, 10.1 mmol).

The crude product was purified by recrystallising twice from ethyl acetate-hexane. Yield: 2.45 g (33%). 1 H-NMR; δ ((CD₃)₂SO), 8.44 (1H, d, J = 9.3 Hz), 8.20 (1H, d, J = 7.8 Hz), 7.89 (1H, d, J = 9.3 Hz), 7.67 (1H, br m), 5.70 - 5.63 (1H, m), 5.18 - 4.95 (4H, m), 4.81 (1H, t, J = 9.8 Hz), 4.22 - 4.14 (2H, m), 3.97 - 3.79 (4H, m), 2.58 (3H, d, J = 4.5 Hz), 2.33 - 2.27 (1H, m), 2.20 - 2.02 (5H, m), 1.99 (3H, s), 1.96 (3H, s), 1.91 (3H, s), 1.90 - 1.85 (2H, m), 1.73 (3H, s), 1.55 - 1.46 (1H, m), 0.84 (3H, d, J = 6.4 Hz), 0.78 (3H, d, J = 6.6 Hz).

Example 5c

3S-Allyl-4-hydroxy-2R-isobutylsuccinyl-N⁴-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- α -D-glucopyranosyl)-L-glutamine-N-methylamide

[3S-Allyl-4-*tert*-butoxy-2R-isobutylsuccinyl]-N⁴-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- α -D-glucopyranosyl)-L-glutamine-N-methylamide (2.2 g, 3.0 mmol) was deprotected by TFA acidolysis, as described in Example 1d.

Recrystallisation from methanol / diisopropyl ether afforded the title compound as a white solid. Yield: 950 mg (47%). 1 H-NMR; δ ((CD₃)₂SO), 8.44 (1H, d, J = 9.3 Hz), 8.19 (1H, d, J = 7.7 Hz), 7.89 (1H, d, J = 9.1 Hz), 7.65 (1H, br m), 5.75 - 5.64 (1H, m), 5.18 - 4.94 (4H, m), 4.81 (1H, t, J = 9.8 Hz), 4.20 - 4.14 (2H, m),

3.97 - 3.80 (4H, m), 2.58 (3H, d, $J = 4.5$ Hz), 2.40 - 2.32 (1H, m), 2.19 - 1.90 (5H, m), 1.99 (3H, s), 1.96 (3H, s), 1.91 (3H, s), 1.73 (3H, s), 1.58 - 1.38 (2H, m), 0.95 (1H, m), 0.83 (3H, d, $J = 6.5$ Hz), 0.78 (3H, d, $J = 6.6$ Hz).

Example 5d

[3S-Allyl-4-(N-hydroxyamino)-2R-isobutylsuccinyl]-N⁴-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- α -D-glucopyranosyl)-L-glutamine-N-methylamide

To a solution of [3S-allyl-4-hydroxy-2R-isobutylsuccinyl]-N⁴-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- α -D-glucopyranosyl)-L-glutamine-N-methylamide (920 mg, 1.35 mmol) in DMF (20 ml) was added HOBr (200 mg, 1.48 mmol) EDC (335 mg, 1.75 mmol) and the mixture was stirred for 90 minutes at room temperature. Hydroxylamine hydrochloride (111 mg, 1.60 mmol) was added followed by NMM (177 mg, 1.75 mmol) and stirring was continued overnight. The solvent was removed under reduced pressure. Trituration with diethyl ether and water gave a white solid which was collected by filtration and recrystallised from methanol / diisopropyl ether. Yield: 675 mg (72%).

¹H-NMR; δ ((CD₃)₂SO), 10.4 (1H, s), 8.71 (1H, s), 8.45 (1H, d, $J = 9.6$ Hz), 8.13 (1H, d, $J = 7.8$ Hz), 7.88 (1H, br m), 5.61 - 5.58 (1H, m), 5.16 - 4.78 (4H, m), 4.81 (1H, t, $J = 9.8$ Hz), 4.20 - 4.15 (2H, m), 3.97 - 3.80 (4H, m), 2.57 (3H, d, $J = 4.4$ Hz), 2.24 - 2.05 (5H, m), 1.99 (3H, s), 1.96 (3H, s), 1.90 (3H, s), 1.75 (1H, m), 1.73 (3H, s), 1.50 (2H, m), 0.98 - 0.95 (1H, m), 0.81 (3H, d, $J = 6.5$ Hz), 0.77 (3H, d, $J = 6.3$ Hz).

Example 5e

[3S-Allyl-4-(N-hydroxyamino)-2R-isobutylsuccinyl]-N⁴-(2-acetamido-2-deoxy- α -D-glucopyranosyl)-L-glutamine-N-methylamide

[3S-Allyl-4-(N-hydroxyamino)-2R-isobutylsuccinyl]-N⁴-(2-acetamido-3,4,6-tri-O-

acetyl-2-deoxy- α -D-glucopyranosyl)-L-glutamine-N-methylamide (650 mg, 0.93 mmol) was O-deacetylated as described in Example 1e. The product was recrystallised from methanol-diisopropyl ether. Yield: 251 mg (47%). m.p. 232-234°C. 1 H-NMR; δ ((CD₃)₂SO), 10.42 (1H, s), 8.13 (1H, d, J = 7.9 Hz), 7.95 (1H, d, J = 8.9 Hz), 7.77 (1H, d, J = 4.7 Hz), 5.60 (1H, m), 4.94 (2H, m), 4.80 (1H, t, J = 9.3 Hz), 4.20 (1H, m), 3.66 - 3.30 (5H, m), 3.08 (2H, d, J = 6.4 Hz), 2.58 (3H, d, J = 4.5 Hz), 2.24 - 1.99 (4H, m), 1.96 - 1.91 (1H, m), 1.86 - 1.70 (4H, m and s), 1.45 - 1.39 (2H, m), 0.97 - 0.93 (1H, m), 0.82 (3H, d, J = 6.4 Hz), and 0.77 (3H, d, J = 6.3 Hz). 13 C-NMR; δ ((CD₃)₂SO), 173.5, 172.1, 171.5, 170.2, 169.4, 135.9, 116.4, 79.2, 78.8, 74.6, 70.8, 61.1, 54.8, 52.4, 46.1, 45.9, 34.8, 32.3, 27.9, 25.6, 25.3, 24.2, 22.9 and 21.6.

Biological Example A

The potency of compounds of the invention as inhibitors of collagenase was determined by the procedure of Cawston and Barrett, (Anal. Biochem., **99**, 340-345, 1979), hereby incorporated by reference, whereby a 1mM solution of the compound being tested, or a dilution thereof, was incubated at 37°C for 16 hours with collagen and collagenase (buffered with 25mM Hepes, pH 7.5 containing 5mM CaCl₂, 0.05% Brij 35 and 0.02% NaN₃). The collagen was acetylated ¹⁴C collagen prepared by the method of Cawston and Murphy, (Methods in Enzymology, **80**, 711, 1981), hereby incorporated by reference. The samples were centrifuged to sediment undigested collagen, and an aliquot of the radioactive supernatant removed for assay on a scintillation counter as a measure of hydrolysis. The collagenase activity in the presence of 1mM of the test compound, or a dilution thereof, was compared to activity in a control devoid of inhibitor and the result reported below as that of inhibitor concentration effecting 50% inhibition of the collagenase activity (IC₅₀).

The potency of compounds of the invention as inhibitors of stromelysin was determined by the procedure of Cawston et al, (Biochem. J., **195**, 159-165,

1981), hereby incorporated by reference, whereby a 1mM solution of the compound being tested, or a dilution thereof, was incubated at 37° for 16 hours with stromelysin and ^{14}C acetylase casein (buffered with 25mM Hepes, pH 7.5 containing 5mM CaCl_2 , 0.05% Brij 35 and 0.02% NaN_3). The casein was acetylated ^{14}C casein prepared by the method of Cawston et al (ibid). The stromelysin activity in the presence of 1mM of the test compound, or a dilution thereof, was compared to activity in a control devoid of inhibitor and the result reported below as that of inhibitor concentration effecting 50% inhibition of the stromelysin activity (IC_{50}).

Results:

Compound	Collagenase IC_{50} (nM)	Stromelysin IC_{50} (nM)
Example 3	20	600

Biological Example B

The ability of example compounds of the invention to inhibit the release of TNF was investigated. The assay is based on the ability of phorbol myristate acetate (PMA) to stimulate the release of TNF α from a human monocytic cell line, U937.

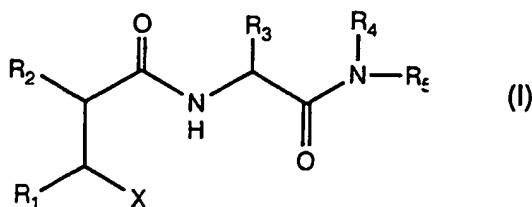
U937 cells cultured in RPMI 1640 medium + 5% foetal calf serum are centrifuged at 1000 x g for 5 minutes and then resuspended in medium at 2 x 10^6 / ml. 1 ml of cell suspension is aliquoted into individual wells of 24-well plates. Test compounds are dissolved in dimethyl sulphoxide (DMSO) at a stock concentration of 100mM, then diluted to 50x the final required concentration with RPMI 1640 medium. 20 μ l of the diluted compounds are added to U937 cells in duplicate wells. TNF α release is stimulated by addition of PMA to the cells at a final concentration of 50nM. Appropriate control cultures are set up in duplicate. The plates are incubated for 18 hours at 37°C,

5% CO₂, then centrifuged at 1000 x g for 5 minutes. A specific ELISA for TNF α obtained from R&D Systems Europe Ltd, Abingdon, England is used to measure TNF α levels in the culture supernatants

The average concentration of test compound which inhibits the release of TNF α by 50% relative to the control culture was assessed. The compound of example 5 above had IC₅₀ value less than 3.5 μ M. μ M.

Claims

1. A compound of general formula (I):



wherein;

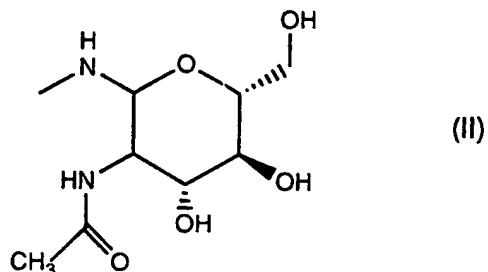
X is -CONHOH or -CO₂H;

R₁ is hydrogen, a C₁-C₆ alkyl, C₂-C₆ alkenyl, phenyl, substituted phenyl, phenyl(C₁-C₆ alkyl), heterocycl, substituted heterocycl, heterocycl(C₁-C₆ alkyl), substituted heterocycl(C₁-C₆ alkyl), or a group BSO_nA- wherein n is 0, 1 or 2 and B is hydrogen or a (C₁-C₆) alkyl, phenyl, substituted phenyl, heterocycl, C₁-C₆ acyl, phenacyl or substituted phenacyl group, and A represents C₁-C₆ alkyl; amino; protected amino; acylamino; OH; SH; C₁-C₆ alkoxy; C₁-C₆ alkylamino; C₁-C₆ alkylthio; aryl(C₁-C₆ alkyl); amino(C₁-C₆ alkyl); hydroxy(C₁-C₆ alkyl), mercapto(C₁-C₆ alkyl) or carboxy(C₁-C₆ alkyl) wherein the amino-, hydroxy-, mercapto- or carboxyl-group are optionally protected or the carboxyl- group amidated; lower alkyl substituted by maleimido, succinimido, naphthalimido, 2,3-dihydro-1,3-dioxo-1H-benz[d,e]isoquinol-2-yl, carbamoyl, mono(lower alkyl)carbamoyl, di(lower alkyl)carbamoyl, di(lower alkyl)amino, carboxy-lower alkanoylamino, pyrrolidino or morpholino;

R₂ is a (C₁-C₆)alkyl, (C₂-C₆)alkenyl, (C₂-C₆)alkynyl, phenyl(C₁-C₆)alkyl,

heteroaryl(C₁-C₆)alkyl, cycloalkyl (C₁-C₆)alkyl or cycloalkenyl (C₁-C₆)alkyl group anyone of which may be optionally substituted by one or more substituents selected from (C₁-C₁₀)alkyl, O(C₁-C₆) alkyl, S(C₁-C₆) alkyl, O(C₁-C₆ alkyl)OC₁-C₆ alkyl, S(C₁-C₆ alkyl)OC₁-C₆ alkyl, O(C₁-C₆ alkyl)SC₁-C₆ alkyl or S(C₁-C₆ alkyl)SC₁-C₆ alkyl;

R₃ is (a) a group -(C₁-C₆ alkyl)COR₆, or -(C₁-C₆ alkyl)(C₆H₄)XR₆ where X is a group -OCH₂CO-, -CO-, -CH₂CH₂CO- or -NHCH₂CO- and R₆ is a group of formula (II):



or (b) (subject to the proviso below) the side chain of a naturally occurring amino acid, which may be protected if functional groups are present, eg by acylation of amino groups and amidation of carboxyl groups; or a group CR₈R₉R₁₀ in which each of R₈, R₉ and R₁₀ is independently hydrogen, (C₁-C₆)alkyl, (C₂-C₆)alkenyl, (C₂-C₆)alkynyl, phenyl(C₁-C₆)alkyl, hydroxyl, O(C₁-C₆) alkyl, S(C₁-C₆) alkyl, OPh, OCH₂Ph, SPh, SCH₂Ph, halogen, CN, CO₂H, (C₁-C₄)perfluoroalkyl, CH₂OH, CO₂(C₁-C₆)alkyl, or a group phenyl or heteroaryl which is optionally substituted by one or more substituents independently selected from hydrogen, hydroxyl, halogen, CN, CO₂H, CO₂(C₁-C₆)alkyl, CONH₂, CONH(C₁-C₆)alkyl, CONH(C₁-C₆alkyl)₂, CHO, CH₂OH, (C₁-C₄)perfluoroalkyl, O(C₁-C₆)alkyl, S(C₁-C₆)alkyl, SO(C₁-C₆)alkyl, SO₂(C₁-

C_6 alkyl, NO_2 , NH_2 , $NH(C_1-C_6)$ alkyl, $N((C_1-C_6)$ alkyl) $_2$, $NHCO(C_1-C_6)$ alkyl, (C_1-C_6) alkyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, (C_3-C_8) cycloalkyl, (C_4-C_8) cycloalkenyl, phenyl or benzyl; or R_8 and R_9 together with the carbon atom to which they are attached form a 3 to 8 membered cycloalkyl or a 5- to 6-membered heterocyclic ring; or R_8 , R_9 and R_{10} together with the carbon atom to which they are attached form a bicyclic ring (for example adamantyl);

R_4 is (i) a group $-(CH(R_7)CONH)_mCOR_6$, wherein $m = 0, 1$, or 2 , R_6 is as defined above, and R_7 is hydrogen or the side chain of a naturally occurring amino acid, which may be protected if functional groups are present, eg by acylation of amino groups and amidation of carboxyl groups; or (ii) (subject to the proviso below) hydrogen, C_1-C_6 alkyl, (C_1-C_4) perfluoroalkyl or a group $D-(C_1-C_6$ alkyl) wherein D represents hydroxy, (C_1-C_6) alkoxy, (C_1-C_6) alkylthio, acylamino, optionally substituted phenyl or heteroaryl, NH_2 , or a mono- or di- (C_1-C_6) alkyl amine;

R_5 is hydrogen or a (C_1-C_6) alkyl group;

provided that at least one of the groups R_3 and R_4 contains a group R_6 as defined above

or a salt, hydrate or solvate thereof.

2. A compound as claimed in claim 1 wherein R_4 is a group $-(CH(R_7)CONH)_mCOR_6$, wherein $m = 0, 1$, or 2 , R_6 is as defined in claim 1, and R_7 is hydrogen.
3. A compound as claimed in claim 1 or claim 2 wherein R_1 is hydrogen,

hydroxyl or allyl.

4. A compound as claimed in any of the preceding claims wherein R₂ is isobutyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, cyclohexylpropyl, cyclohexylbutyl, cyclohexylpentyl, phenylethyl, phenylpropyl, phenylbutyl, phenylpentyl, propyloxymethyl or propylsulphanyl.

5. A compound as claimed in any of the preceding claims wherein R₃ is benzyl or t-butyl.

6. A compound as claimed in any of claims 1-4 wherein R₄ is methyl, ethyl, propyl, butyl, hydroxyethyl, hydroxypropyl, 2,2-dimethyl-3-hydroxypropyl, hydroxybutyl, methoxyethyl, ethoxyethyl, methoxypropyl, 2,2-dimethyl-3-methoxypropyl, 2,2-dimethyl-3-ethoxypropyl, 2-ethylthioethyl, 2-acetoxyethyl, N-acetyl-aminoethyl, 3-(2-pyrrolidone)propyl, optionally substituted phenylethyl, phenylpropyl, phenylbutyl and phenylpentyl.

7. A compound as claimed in any of the preceding claims wherein R₅ is hydrogen.

8. A compound selected from the group consisting of;

[4-(N-Hydroxyamino)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycanyl-N-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-amide;

[4-(N-Hydroxyamino)-2R-isobutylsuccinyl]-L-phenylalaninyl-glycanyl-glycanyl-N-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-amide;

[4-(N-Hydroxyamino)-2R-isobutylsuccinyl]-O-[(2-acetamido-2-deoxy- β -D-glucopyranosyl)carboxamidomethyl]-L-tyrosine-N-methylamide;

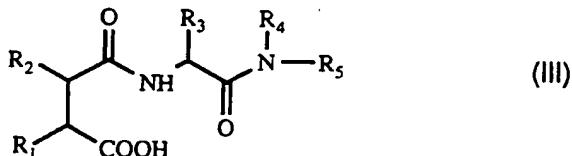
3S-Hydroxy-4-(N-hydroxyamino)-2R-isobutylsuccinyl-L-phenylalaninyl-

glycinyl-N-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-amide;
and salts, solvates and hydrates thereof.

9. [3S-Allyl-4-(N-hydroxyamino)-2R-isobutylsuccinyl]-N⁴-(2-acetamido-2-deoxy- α -D-glucopyranosyl)-L-glutamine-N-methylamide and salts, solvates and hydrates thereof.

10. A process for the preparation of a compound as claimed in claim 1 in which X is a hydroxamic acid group (-CONHOH), which process comprises:

(a) coupling an acid of general formula (III)

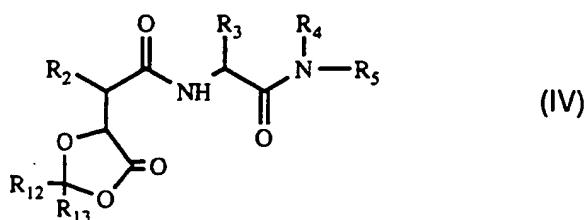


or an activated derivative thereof with hydroxylamine, O-protected hydroxylamine, or a salt thereof, R_1 , R_3 , R_4 , and R_5 being as defined in general formula (I) except that any substituents in R_1 , R_2 , R_3 , R_4 , and R_5 which are potentially reactive with hydroxylamine, O-protected hydroxylamine or their salts may themselves be protected from such reaction, then removing any protecting groups from the resultant hydroxamic acid moiety and from any protected substituents in R_1 , R_2 , R_3 , R_4 , and R_5 ; and

(b) optionally converting a compound of general formula (I) into another compound of general formula (I).

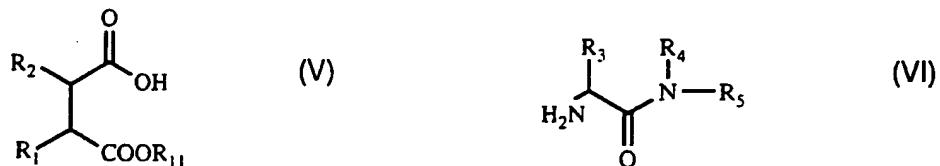
11. A process as claimed in claim 10 wherein an activated derivative of a compound of formula (III) is used and said activated derivative is a pentafluorophenyl, hydroxysuccinyl, or hydroxybenztriazyl ester.

12. A process as claimed in claim 10 or claim 11 wherein (in the special case where R_1 in compound (I) is hydroxy) the hydroxy group R_1 and the adjacent carboxyl group are simultaneously protected as a dioxalone of formula (IV):



wherein the groups R_{12} and R_{13} are derived from a dioxalone forming reagent, and the dioxalone ring being is opened by the reaction with hydroxylamine to give the required hydroxamic acid derivative of formula (I).

13. A process for the preparation of a compound as claimed in claim 1 in which X is a carboxylic acid group (-COOH) which process comprises coupling an acid of formula (V) or an activated derivative thereof with an amine of formula (VI)



wherein R_1 , R_2 , R_3 , R_4 , and R_5 are as defined in general formula (I) except that any substituents in R_1 , R_2 , R_3 , R_4 , and R_5 which are potentially reactive in the coupling reaction may themselves be protected from such reaction, and R_{11} represents a hydroxy protecting group, and subsequently removing the protecting group R_{11} and any protecting groups from R_1 , R_2 , R_3 , R_4 , and R_5 .

14. A process as claimed in claim 13 wherein an activated derivative of a

compound of formula (V) is used and said activated derivative is a pentafluorophenyl ester, acid anhydride or acid halides, eg chloride.

15. A process as claimed in claim 13 or claim 14 wherein (in the special case where R₁ in compound (I) is hydroxy) compound (V) has the formula (VII):



wherein R₂ is as defined in general formula (I) and the groups R₁₂ and R₁₃ are derived from a dioxalane forming reagent.

16. A process as claimed in claim 12 or claim 15 wherein the groups R₁₂ and R₁₃ are hydrogen, alkyl, phenyl or substituted phenyl.

17. A method of management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF in mammals including humans, which method comprises administering to the mammal an effective amount of a compound as claimed in any one of claims 1 to 9.

18. A compound as claimed in any one of claims 1 to 9 for use in human or veterinary medicine, in the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF.

19. The use of a compound as claimed in any one of claims 1 to 9 in the preparation of an agent for the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF.

21. A method as claimed in claim 17, a compound for use as claimed in claim 18, or the use as claimed in claim 19, wherein the diseases or condition referred to is one mediated by an MMP.
22. A method as claimed in claim 17, a compound for use as claimed in claim 18, or the use as claimed in claim 19, wherein the diseases or condition referred to is rheumatoid arthritis, osteoarthritis, periodontitis, gingivitis, corneal ulceration or tumour invasion by secondary metastases.
23. A method as claimed in claim 17, a compound for use as claimed in claim 18, or the use as claimed in claim 19, wherein the diseases or condition referred to is one mediated by TNF.
24. A method as claimed in claim 17, a compound for use as claimed in claim 18, or the use as claimed in claim 19, wherein the disease or condition referred to is inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, an acute infection, a shock state, a graft versus host reaction or autoimmune disease.
25. A pharmaceutical or veterinary composition comprising a compound as claimed in any one of claims 1 to 9 together with a pharmaceutically or veterinarilly acceptable excipient or carrier.
26. A pharmaceutical or veterinary composition as claimed in claim 25 which is adapted for oral administration.
27. A compound as claimed in claim 1 except that one or more of the hydroxy groups in the group R₆ is protected.
28. A compound as claimed in claim 27 in which the said hydroxy groups are protected as the acetate.

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/GB 94/00808

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07H13/04 C07K9/00 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07H C07K A61K C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 489 577 (CELLTECH LIMITED) 10 June 1992 cited in the application see the whole document ----	1,8,10, 13, 17-19, 25-27
P,A	WO,A,94 02447 (BRITISH BIO-TECHNOLOGY LIMITED) 3 February 1994 see the whole document -----	1,8,10, 13, 17-19, 25-27

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *'A' document defining the general state of the art which is not considered to be of particular relevance
- *'E' earlier document but published on or after the international filing date
- *'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *'O' document referring to an oral disclosure, use, exhibition or other means
- *'P' document published prior to the international filing date but later than the priority date claimed

- *'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *'&' document member of the same patent family

2

Date of the actual completion of the international search 21 July 1994	Date of mailing of the international search report 03.08.94
Name and mailing address of the ISA European Patent Office, P.O. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Moreno, C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB94/00808

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 17, 21-24

because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 17 and 21 to 24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte: **inal Application No**

PCT/GB 94/00808

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0489577	10-06-92	AU-A-	9017391	25-06-92
		AU-A-	9023391	25-06-92
		EP-A-	0489579	10-06-92
		WO-A-	9209564	11-06-92
		WO-A-	9209565	11-06-92
		GB-A-	2255339	04-11-92
		GB-A-	2255340	04-11-92
		HU-A-	61973	29-03-93
		JP-T-	5503719	17-06-93
		JP-T-	5503720	17-06-93
		US-A-	5300501	05-04-94
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WO-A-9402447	03-02-94	AU-B-	4715293	14-02-94
		AU-B-	4715393	14-02-94
		WO-A-	9402446	03-02-94
		GB-A-	2268933	26-01-94
		GB-A-	2268934	26-01-94
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